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Utilization of Defatted and Phenolic-Extracted Grape Pomace Flour in Bakery Products: Valorization and Functional Potential

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Abstract: Grape pomace is a high-volume by-product of wine production, whose thorough valorization is of particular importance for the modern circular bioeconomy and the sustainable development of the agri-food sector. The present study aims to evaluate the potential of flour obtained from grape pomace for application in bakery products. After the removal of oil and the main phenolic fraction, the resulting flour is characterized by a high dietary fiber content and is considered a promising raw material for improving the structural parameters of dough and the functional–technological properties of the final products. The incorporation of such flour into bakery formulations offers the possibility to simultaneously reduce reliance on conventional flours and increase the intake of dietary fiber and bioactive components, which is in line with current scientific and technological trends in the development of functional and sustainable food products. This approach, on the one hand, highlights the specific technological potential of defatted and phenolic-extracted grape pomace flour and, on the other hand, strengthens the integration of wine-making by-products into the value chain as a source of high value-added ingredients for the food industry.

Keywords: Circular bioeconomy, Grape pomace flour, Valorization

Introduction

Grapes are among the most widely cultivated crops worldwide, with global production estimated at over 79 million tons in 2018, according to the Food and Agriculture Organization (FAO) of the United Nations. Consumption of grapes has been associated with beneficial effects on human health owing to their high content of bioactive compounds. Approximately 75% of the grapes produced globally are dedicated to wine production, of which about 20–30% correspond to by-products. These residues are commonly referred to as grape pomace and consist primarily of skins, residual pulp, seeds, and stalks (Flamini et al.,2013; Rodriguez-Lopez et al.,2022).

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The management of grape pomace waste poses a significant environmental challenge. Concurrently, consumer demand is rising for natural, health-oriented food ingredients that can supplant synthetic antioxidants and preservatives. The biotechnological potential of grape pomace has spurred extensive research exploring its utility as a fortification agent in food products (Castaldo et al., 2019; Flamini et al.,2013; Rodriguez-Lopez et al.,2022).

In nutraceuticals, the polyphenolic compounds and dietary fiber in grape pomace (GP), and especially in grape seeds underpin its antioxidant, anti-inflammatory, and metabolic health benefits, enabling applications as antioxidant supplements, gut health promoters, weight management aids, and cardiovascular protectants. Although challenges persist regarding sensory modification and bioavailability optimization, GP's versatility and sustainability underscore its potential for innovative, health-focused product development (López-Alarcón & Denicola, 2013; Vanidze et al., 2025; Zhishen et al., 1999).

The escalating global demand for sustainable and innovative solutions in the food and pharmaceutical sectors has intensified efforts to valorize agricultural residues. Winemaking by-products, previously deemed valueless, have attracted considerable interest due to their rich nutritional composition and health-promoting potential. Research has established that these materials are replete with antioxidants, polyphenols, dietary fibers, and other bioactive compounds, which confer diverse benefits such as anti-inflammatory, anticancer, and cardioprotective effects (Castaldo et al., 2019; Rodriguez-Lopez et al., 2022; Vanidze et al., 2025; Zhishen et al., 1999).

The proportion of seeds retained in grape pomace following press extraction is influenced by cultivar, maturation stage, and pressing methodology. On average, seeds constitute approximately 20–25% of the total pomace mass. When the pomace is considered excluding the rachis and skin fraction (i.e., the lignocellulosic skeleton), the relative contribution of seeds increases to approximately 40–50% of the residual mass (Re et al., 1999).

Particular emphasis has been placed on grape seeds as a source of diverse bioactive compounds. Recent research has evinced growing interest in wine production residues—particularly pomace—and their active incorporation into the food industry to enhance product nutritional value. Grape seeds: 35-45% fibers, 10-20% fat, 8-13 % proteins, 5-10% phenols (Flamini et al., 2013; Rodriguez-Lopez et al., 2022).

Study Objective

Incorporation of grape seeds flour (GSF)—derived from Saperavi wine production residues—into wheat flour for bread production, followed by comprehensive analysis of the resultant product.

1. To produce flour from grape seeds.
2. Comparing the quality attributes of a confectionery product formulated with grape seed flour against a wheat flour-based counterpart, thereby identifying its advantages.

Materials

Saperavi grape seeds were collected from the Kakheti region of Eastern Georgia during the 2024 harvest season.

Bread sample preparation

Saperavi grape seeds flour (GSF) (Figure 2) was the third and final fraction of a three-step extraction process: in stage I, the lipid fraction was isolated by mechanical cold-press extraction; in stage II, the phenolic fraction was isolated using ethanol; and in stage III, flour with a high concentration of dietary fiber was obtained, which was used as a functional and low-calorie food ingredient. This approach is consistent with the principle of a circular bioeconomy, where all side streams are integrated into the value chain (Boff et al., 2022; Souza et al., 2024).

Two types of bread were used in the study: a control sample made from 100% wheat flour, and an experimental sample with 10% Saperavi grape seed flour (GSF) (Figure 1). The following parameters were measured: 1. pH, titratable acidity, moisture (Table 1); 2. specific volume and porosity (Table 2); 3. total phenols and flavonoids (Table 3); antioxidant activity by DPPH and ABTS methods (Table 4); 4. the crude protein content in GSF.

Figure 1. GSF

Figure 2. GSF Bread

Methods:

1.1 Method for Determining pH (Active Acidity)

The active acidity (pH) of bread was determined by the potentiometric method according to AACC International Method 02-52.01(AACC International, n.d.). For this, 10 g of well-ground bread sample was placed in a beaker and 90 ml of distilled water was added. The resulting suspension was mixed for 5 minutes, after a 10-minute delay the pH was measured using a METTLER TOLEDO pH meter. Calibration was performed with standard buffer solutions (pH 4.00 and 7.00). Each sample was measured in triplicate; the result is presented as an average value (Tolve et al., 2021)

1.2 Titratable Acidity Determination Method

Titrate acidity was determined by titrimetric method according to ISO 7230:2007 (International Organization for Standardization, 2007). 10 g of ground bread sample was added to 90 ml of distilled water and intensive mixing was carried out for 5 min. The suspension was filtered; 50 ml of the filtrate was used for titration. 2–3 drops of phenolphthalein indicator (1% ethanolic solution) were added to the sample and titration was carried out with 0.1 N NaOH solution until a faint pink color appeared, which was stable for 30 s. Titrated acidity was calculated as the percentage equivalent of lactic acid according to the following formula (Tolve et al., 2021):

$$\% = (V \times 0.1 \times 0.090 \times 2) / m \times 100$$

Where: V — volume of 0.1 N NaOH used for titration (ml); 0.090 — thousandths of the molar mass of lactic acid (g); 2 — dilution factor (50 ml of 100 ml is used); m — sample mass (g).

1.3 Moisture Determination Method

The moisture content of bread was determined gravimetrically according to AACC International Method 44-19.01 (AACC International, n.d.). Approximately 5 g of bread crumb sample was weighed into a pre-weighed container and placed in an oven at 105 ± 2°C. The sample was dried to constant weight (3–4 hours). The moisture content was calculated using the following formula (Tolve et al., 2021):

$$\text{Moisture } (\%) = (m_1 - m_2) / m_1 \times 100$$

Where: m_1 — mass of sample before drying (g); m_2 — mass of sample after drying (g).

Each sample was analyzed in triplicate.

2.1 Determination of Bread Specific Volume:

The specific volume of bread was determined using the seed displacement method according to AACC International Method 10-05.01 (AACC International, n.d.). In this method, a bread sample was placed in a half-filled container filled with rapeseed. The difference in seed volume indicated the space occupied by the bread. The specific volume was calculated using the formula (AACC International, n.d.; Tolve et al., 2021):

$$SV = V / m$$

Where: SV — specific volume of bread (cm^3/g); V — volume of bread (cm^3), determined by seed displacement; m — mass of bread (g), determined by analytical balance.

2.2 Determination of Bread Porosity

Bread porosity was determined by the geometric density method (Sapirstein et al., 2007; Tolve et al., 2021). A cylindrical sample with standard parameters was cut from the bread crumb: diameter 3 cm, height 3 cm; since the volume of the cylinder $V = \pi \times r^2 \times h = \pi \times 1.5^2 \times 3 \approx 21.2$ cm^3. The mass of the sample (m) was determined using an analytical balance. The density of the sample was calculated:

$$\rho = m / V$$

Bread porosity was calculated by the following formula (Sapirstein et al., 2007):

$$P = (1 - \rho / \rho_0) \times 100$$

Where: P — porosity (%); ρ — bread crumb density (g/cm^3); ρ_0 — the conditional density of the solid phase of bread, taken equal to 1.27 g/cm^3 — this is a conventional value, widely found in the literature (Besbes et al., 2013; Sapirstein et al., 2007).

3.1. Total Phenols — Folin-Ciocalteu

The total content of phenolic compounds was determined by the standard Folin-Ciocalteu spectrophotometric method. Folin-Ciocalteu reagent (1 ml) and Na_2CO_3 solution (2 ml) were added to 1 ml of the sample extract; incubation was carried out for 1 h at room temperature. The optical density was determined at 760 nm. The results were converted to mg GAE/100 g, on a dry sample (Ieri et al., 2021; Tolve et al., 2021).

3.2. Total Flavonoids — $AlCl_3$

The determination of flavonoids was carried out by the $AlCl_3$ spectrophotometric method [20]. 1 ml extract + 5 ml H_2O + 0.3 ml 5% $NaNO_2$ (5 min) + 0.3 ml 10% $AlCl_3$ (6 min) + 2 ml 1N NaOH. Op. density — 510 nm. Calibration — quercetin. Results: mg QE/100g, on dry basis (Oprea et al., 2022; Zhishen et al,1999).

3.3. Antioxidant Activity — DPPH IC_{50}

DPPH (2,2-diphenyl-1-picrylhydrazyl) method according to Brand-Williams et al. (1995) Extracts of different concentrations were mixed with 0.1 mM DPPH solution; incubation for 30 min in the dark, room temperature. Op. density — 517 nm. IC_{50} — min. sample mass required for 50% inhibition (mg). Low IC_{50} = strong antioxidant. Results — on dry basis (Hoye & Ross, 2011).

3.4. Antioxidant Activity — ABTS IC_{50}

$ABTS^{\bullet+}$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulf. mj.)) method according to Re et al. [15]. $ABTS^{\bullet+}$ — ABTS 7 mM + $K_2S_2O_8$ 2.45 mM, 12–16 h in the dark. The solution is diluted to $OD_{734} = 0.70 \pm 0.02$. Extract + $ABTS^{\bullet+}$ → inc. 6 min → OD 734 nm. IC_{50} — mg, 50% inhib., dry base. ABTS is sensitive to hydrophilic antioxidants, DPPH — lipophilic; therefore, a combination of the two tests gives a complete picture (Lopez-Alarcon & Denicola, 2013; Re et al., 1999).

4.1. The Crude Protein Content Was Determined by the Eldar Method

The crude protein content was determined by the Eldar method in accordance with the official method of AOAC International (AOAC 976.05) (AOAC International, 2005; Mariotti et al., 2008). The crude protein content was calculated at the expense of total nitrogen (N × 6.25), and the results were expressed as mass on a dry basis (% d.w.).

Results:

Table 1. pH, titratable acidity and moisture

Parameters	White bread (control sample)	GSF bread (+10% seeds flour)
pH active acidity	5,80	5,26
Titratable acidity (%)	0,24	0,32
Moisture (%)	27,84	28,03

pH active acidity - The GSF-enriched bread was characterized by a statistically significant decrease in pH from 5.80 to 5.26 ($\Delta = -0.54$ units) compared to the control sample. Given the logarithmic nature of pH, this 0.54 unit decrease corresponds to a 3.47-fold increase in the H^+ concentration of the aqueous phase ($100.54 = 3.47$). This change is due to the acidic nature of the phenolic compounds contained in GSF - namely gallic acid (pKa = 4.4), ellagic acid (pKa = 4.0) and condensed tannins. These compounds, despite extraction, remain partially covalently bound to the fiber matrix, are distilled during baking heat treatment and reduce pH (Sporin et al., 2018). The pH of the control bread, 5.80, is at the lower end of the normative range (standard white bread: pH 5.8–6.2), while the pH of the GSF bread, 5.26, is within the typical range for functional bread (4.8–5.5) (Hoye & Ross, 2011). This pH range is technologically advantageous, as it helps to increase microbiological stability and optimize fiber hydration.

Titratable acidity - The addition of 10% GPF increases the titratable acidity from 0.24% (2.7°) to 0.32% (3.6°) - 1.33 times. It is noteworthy that the decrease in pH ($H^+ \times 3.47$) significantly exceeds the increase in titratable acidity (×1.33). This asymmetry is due to the buffering effect of GSF: grape seed proanthocyanidins and tannins, due to their polymeric nature, effectively release H^+ (pH ↓↓) (Boff et al., 2022; Castaldo et al., 2019). The obtained values are within the standard: control 2.7° ≤ GOST 27842 ≤ 3.0°; GPF bread 3.6° ≤ GOST 2077 ≤ 4.0° (functional bread).

Moisture - The moisture content of GSF bread (28.03%) increased by +0.19 pp compared to the control sample (27.84%). This difference, despite its small size, is technologically important: it is due to the hygroscopic nature of the insoluble dietary fiber of GSF - the fiber replaces gluten proteins during dough formation and reacts with water, increasing the water absorption capacity and retaining more bound water after baking (Iuga & Mironeasa, 2020). In addition, this hydrating effect of fiber has a direct impact on the formation of the gluten network: the GSF particles physically disrupt the continuity of the gluten network - a dilution effect on the protein matrix - which reduces the elasticity of the dough (Iuga & Mironeasa, 2020; Souza et al., 2024).

Table 2. Specific volume, porosity

Parameters	White bread (control sample)	GSF bread (+10% seeds flour)
Specific volume (cm^3/g)	2,08	1,87
Porosity (%)	61,2	58,2

The inclusion of 10% GSF leads to a 10.1% decrease in the *specific volume* of the bread (from 2.08 to 1.87 cm^3/g). This decrease is determined by three interrelated mechanisms. First, the insoluble fiber of GSF

physically disrupts the continuity of the gluten network (dilution effect), reducing the ability of the dough to retain CO_2 during fermentation and baking (Iuga & Mironeasa, 2020). Second, the decrease in pH (from 5.80 to 5.26, Table 1) slightly reduces the metabolic activity of the yeast and, consequently, the intensity of CO_2 production (Sporin et al., 2018). Third, the interaction of phenolic compounds with gluten proteins changes the rheological properties of the dough (Hoye & Ross, 2011). Tolve et al. (2021) reported a similar decrease (from 2.05 to 1.91 cm^3/g) with the addition of 10% grape pomace powder, while Oprea et al. (2022) reported a 7% decrease with the addition of 9% GSF. The specific volume of the baseline control sample (2.08 cm^3/g) is within the normative range for typical white wheat bread (1.8–2.5 cm^3/g), while the GPF bread value (1.87 cm^3/g) remains within this range (Tolve et al., 2021).

Porosity in GSF bread was 58.2% compared to 61.2% in the control (−3.0 pp, −4.9%). This difference is directly attributable to the decrease in specific volume—less volume means fewer air pores in the bread structure. Importantly, the porosity of GPF bread (58.2%) remains well above the minimum normative limit (≥ 54%, GOST 28808), confirming the structural acceptability of the product.

Table 3. Total phenols and flavonoids, mg/100g, calculated on dry basis

Sample	Total phenols mg/100g GAE (dry basis)	Total flavonoids mg/100g QE (dry basis)
White bread (control sample)	35,46	21,75
GSF bread (+10% seeds flour)	50,17	32,07

The total phenolic content of the GSF-enriched bread was 50.17 mgGAE/100g, compared to 35.46 mgGAE/100g in the control sample — an increase of +41.5%. Total flavonoids increased from 21.75 to 32.07 mgQE/100g (+47.4%). The proportionally greater increase in flavonoids confirms that GPF is particularly rich in catechins, epicatechin and condensed proanthocyanidins — the dominant bioactive class in grape seed (Flamini et al., 2013). This increase is particularly significant in the context of the preliminary phenolic extraction of GSF: the content of this fraction belongs to fiber-bound phenols — hydroxycinnamic acids, epicatechin and gallic acid esters — which are covalently bound to the fiber matrix and are not extracted by conventional solvent extraction, although they are partially released during baking heat treatment (160-220 C) (Iuga & Mironeasa, 2020). Tolve et al. (2021) reported a +38% increase in TPC with the addition of 10% grape seeds powder, while Oprea et al. (2022) reported a +45% increase with 9% GSF. Our GSF, a phenolic-extracted flour, gives comparable results to these analogues (+41.5%), which once again confirms the biological importance of fiber-bound phenolic residues.

Table 4. Antioxidant activity — DPPH and ABTS IC_{50}

Sample	Antioxidant activity - 50% inhibition of 0.1 mM DPPH radical by mg of sample on a dry basis	Antioxidant activity - 50% inhibition of ABTS radical using the sample on a dry basis
White bread (control sample)	200,25	177,21
GSF bread (+10% seeds flour)	141,11	105,0

GSF bread showed a statistically significant improvement in antioxidant capacity in both tests. DPPH IC_{50} decreased from 200.25 to 141.11 mg — −29.5% (×1.42). ABTS IC_{50} from 177.21 to 105.0 mg — −40.8% (×1.69). The improvement in ABTS (×1.69) is greater than that in DPPH (×1.42) — this asymmetry is chemically informative: the $ABTS^{\bullet+}$ test is sensitive to hydrophilic antioxidants (phenolic acids, hydroxycinnamates, flavones-3-ols), while DPPH is sensitive to lipophilic components (tocopherols, lipophilic flavonoids). Therefore, GSF provides mainly hydrophilic antioxidant contribution, which is consistent with the profile of fiber-bound hydroxycinnamic acids (coumaric, caffeic, ferric) in grape seed (Iuga & Mironeasa, 2020). The antioxidant improvement directly correlates with the results in Table 3: a +41.5% increase in phenolics is accompanied by a −29.5÷40.8% decrease in IC_{50} (Oprea et al., 2022; Souza et al., 2024; Tolve et al., 2021).

Table 5. The protein content

Sample	GSF	White bread (control sample)	GSF bread (+10% seeds flour)
Protein % (calculated on dry weight)	10	11,23	11,53

The crude protein content in grape seed flour was 10% based on dry weight. This value exceeds the standard norms established for premium wheat flour (11–13 ppm). According to the results, the protein content in standard white bread was 11.23%, while in the GSF bread sample, which was prepared by replacing wheat flakes in bread with grape seed flour (10% mass fraction), the protein content increased to 11.53%.

Discussion

The physicochemical and functional properties of bread fortified with 10% grape seed flour (GSF), a residual fraction from cascade biovalorization of grape pomace, demonstrate coherent alterations attributable to its fiber and residual phenolic components.

Physicochemical Parameters

Incorporation of 10% GSF induces a moderate pH reduction (Δ −0.54), elevated titratable acidity (×1.33), and marginal moisture increase (+0.19 percentage points), all within normative limits. These shifts arise from the acidic nature of bound phenolics and hydrophilic fiber matrix. Concurrently, fiber disrupts gluten networking, phenolic compounds further acidify the matrix, and elevated moisture collectively diminish specific volume (−10.1%) and porosity (−3.0 percentage points), yet remain physicochemically viable.

Functional and Nutritional Enhancements

GSF substitution yields significant bioactive enrichment, including +41.5% total phenolics and augmented antioxidant capacity (DPPH ×1.42; ABTS ×1.69), derived exclusively from fiber-bound residuals released during baking. Protein content interpretation must account for GSF's dual role as a dietary fiber source and gluten-free ingredient; at 10% inclusion, it proportionally dilutes gluten, conferring nutritional relevance for gluten-sensitive consumers, pending targeted gluten quantification. These findings validate GSF as a third valorization stream—post-extraction of oil (stream I) and phenolics (stream II)—for functional bakery applications.

Conclusion

Incorporation of 10% grape seed flour (GSF), a defatted and phenolic-extracted residual from cascade valorization of grape pomace, produces predictable physicochemical modifications while conferring substantive functional and nutritional benefits. GSF inclusion causes a modest acidification, slight moisture increase, and measurable reductions in specific volume and porosity—effects attributable to the fiber-rich, hydrophilic matrix and residual bound phenolics—but these changes remain within acceptable physicochemical limits for bread quality.

Nutritionally, 10% GSF markedly increases dietary fiber and delivers a significant enrichment of total phenolics and antioxidant capacity (DPPH, ABTS), indicating a potential for improved antioxidant status and associated chronic-disease risk reduction when consumed regularly. The partial dilution of gluten by this gluten-free ingredient may have relevance for formulation of products targeted to gluten-sensitive populations, although targeted gluten quantification is required. Overall, GSF represents a viable tertiary valorization stream for grape pomace with clear applicability in functional bakery products; further work should optimize processing and formulation to mitigate texture losses and should assess bioavailability, sensory acceptability, and long-term health outcomes.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

Conflict of Interest

* The authors declare that they have no conflicts of interest.

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